PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P 3098/st	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 02/14511	International filing date *(day/month/year)* 18.12.2002	Priority date *(day/month/year)* 07.01.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/435		
Applicant PETZELT, Christian		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.07.2003	19.05.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - Gitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	Authorized Officer Alconada Rodríguez, Telephone No. +49 30 25901-326

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 02/14511

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-38 as originally filed

Claims, Numbers

1-11 received on 05.05.2004 with letter of 30.04.2004

Drawings, Sheets

1-13 as originally filed

Sequence listing part of the description, pages:

1-8, filed with the letter of 12.05.2003,

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 8, 9 (in part)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 8,9 (in part) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations

   see separate sheet

1 **Re Item III**

1.1 Non-establishment of opinion with regard to novelty, inventive step and industrial applicability.

1.2 Claims 8 and 9 relate to a method for producing a protein in eukaryotic host cells. The claim covers all methods whereby host cells are transfected with a polynucleotide coding for a polypeptide with a deleted or non-functional secretory signal, whereas the application provides support and disclosure for just one such method, namely, the method for expressing a cyplasin from *Aplysia punctata* lacking its signal sequence. The IPEA considers that the teaching in the application of the method for expressing the truncated cyplasin can only be extended to other polypeptides with an amount of experimental effort which amounts to an undue burden for the skilled person. Firstly, the IPEA is not aware, neither from the application nor from common knowledge, of other secreted polypeptides having similar toxicity properties as cyplasin; secondly, for each of these candidate polypeptides, the exact identification of the signal peptide cleavage site should be identified; and thirdly, the method for recovering the truncated polypeptide from the host cells should be optimised on a case by case basis. Therefore, the requirements of Art. 5 PCT are not fulfilled and the claims which refer to said part of the application (**claims 8 and 9**) are also not supported by the application (Art. 6 PCT). Therefore, the subject-matter of **claims 8 and 9** has been examined for those parts which are sufficiently disclosed and supported by the description, namely, those parts relating to the method of expressing a polypeptide consisting of amino acids 20 or 53 to 588 of the Aplysia punctata cyplasin-L (as shown in example 11).

Re Item V

2 Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2.1 Reference is made to the following document:

D1: DATABASE GENEMBL [Online] 21 December 2000 (2000-12-21) PETZELT,C.P.: 'Aplysia punctata mRNA for cyplasin L (ek431 gene)' Database accession no. AJ304802 XP002240886 cited in the application

2.2 Claim 1 relates to a polynucleotide coding for cyplasin with a deleted or non-functional signal sequence is new and involves an inventive step. Document D1 discloses the cyplasin L cDNA which codes for a cytolytic polypeptide of 559 amino acids but it is silent about a cyplasin variant lacking its signal sequence (amino acids 1-52). Furthermore, no mention is done in this document about the possibility of removing or inactivating the signal sequence of cyplasin if an improved expression in mammalian cells is to be achieved. Thus, the subject-matter of **claim 1** is new and involves an inventive step.

2.3 **Claims 2-7 and 10-11**, which refer to the recombinant vectors, host cells, the isolated protein, method for the recombinant production of cyplasin, pharmaceutical compositions and the use of cyplasin for preparing pharmaceutical preparation for treating cancer relate to variations of the known polypeptides as defined in claim 1 and therefore, also relate to new and inventive subject-matter.

2.4 The subject-matter of **claims 8 and 9**, as far as an international preliminary examination report can be carried out (see item 1.1), is new and involves an inventive step.

Claims

1. An isolated nucleic acid molecule encoding the protein cyplasin with a deleted or non-functional secretory signal sequence, being selected from the group consisting of

(a) a nucleic acid molecule encoding a protein comprising the amino acid sequence from position 20 or 53 to position 558 of the sequence marked with "L" of Figure 2(a) (SEQ ID NO:1);

(b) a nucleic acid molecule comprising the sequence of Figure 2(b) (SEQ ID NO:5);

(c) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) or (b) due to the degeneration of the genetic code; and

(d) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a), (b) or (c).

2. A recombinant vector containing a nucleic acid molecule of claim 1.

3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.

4. A recombinant host cell which contains the recombinant vector of claim 2 or 3.

5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

6. An isolated protein encoded by the nucleic acid molecule of

claim 1.

7. A method of making a protein exhibiting biological properties of cyplasin comprising:

(a) culturing the recombinant host cell of claim 4 under conditions such that said protein is expressed; and

(b) recovering said protein.

8. A method of making a cytotoxic protein in eukaryotic host cells which is cytotoxic for said cells when secreted from said cells or externally applied comprising:

(a) culturing a host cell transfected with a nucleic acid sequence encoding said protein with a deleted or non-functional secretory signal sequence under conditions such that said protein is expressed; and

(b) recovering said protein.

9. The method of claim 8 wherein the eukaryotic cells are mammalian cells.

10. A pharmaceutical composition comprising a nucleic acid molecule of claim 1 or a protein of claim 6.

11. Use of a nucleic acid molecule of 1 or a protein of claim 6 for preparing a pharmaceutical composition for treating cancer.